REMARKS

Entry of the foregoing, reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.116, are respectfully requested in light of the remarks which follow.

I. Claim Amendments

By the foregoing amendments to the claims, claim 42 has been amended by deleting the term "about."

The amendments to the claims have been made without prejudice or disclaimer to any subject matter recited or canceled herein. Applicants reserve the right to file one or more continuation and/or divisional applications directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments to the above-identified application are respectfully requested.

II. Response to Claim Rejection Under 35 U.S.C. § 112, Second Paragraph

At pages 2-3 of the Office Action, claim 42 has been rejected under 35 U.S.C. § 112, second paragraph, as purportedly indefinite for reciting "about 20 molecules" of the MPG peptidyl carrier.

In particular, the Examiner has stated that the term "about" is a relative term that does not allow the skilled artisan to quantify the actual numbers of MPG per molecule of antiviral peptide.

To expedite prosecution in the present application, and not to acquiesce to the Examiner's rejection, claim 42 has been amended as described above by deleting the term "about." Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

III. Response to Claim Rejections Under 35 U.S.C. § 103(a)

A. At pages 3-6 of the Office Action, claims 31-38 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Divita et al. (1995) ("Divita et al. 1995") in view of Bolognesi et al. (1995) and Korber et al. (1998).

This rejection is respectfully traversed.

Divita et al. 1995 discloses a synthetic 19-mer peptide, P1, that can inhibit human immunodeficiency virus (HIV) type 2 reverse transcriptase (RT) subunit dimerization in vitro. As recognized by the Examiner, this reference does not disclose decameric peptide inhibitors of RT. Furthermore, Divita et al. 1995 does not demonstrate that the P1 peptide has antiviral activity. Indeed, in Divita et al. 1994 (Divita et al., Inhibition of Human Immunodeficiency virus type 1 reverse transcriptase dimerization using synthetic peptides from the connection domain, Journal of Biological Chemistry, 269:13080-13083 (1994)) the peptide of 19 aa was tested on HIV infected cells, and no activity was shown. This confirms that the peptide of 19 aa does not possess any anti-viral activity. Thus, a person of ordinary skill in the art, in view of Divita et al. 1995, would be inclined to search for other peptides that can inhibit the dimerization of HIV RT.

Finally, the present inventors have suprisingly shown that the reduction of the size of the sequence results in an increase in the inhibition of RT in vitro by a factor of 6. However, nothing in the cited documents would have encouraged one skilled in the art to use a shorter peptide.

Bolognesi et al. relates to a 36-mer peptide which exhibits potent retroviral activity. Such retroviral activity corresponds to the inhibition of HIV transmission to uninfected CD4+ cells. The reference also discloses carboxy or amino truncations of the 36-mer peptide in order to provide peptides of between 3 and 36 amino acid residues, as well as amino acid substitutions, insertions or deletions. Homologs of the 36-mer peptide derived from other HIV-1 and HIV-2 isolates are also provided.

The Examiner considers that the skilled person would have subjected the 19-mer peptide inhibitor of Divita et al. 1995 to further analysis as provided by Bolognesi et al., and would have screened the obtained peptides, thus arriving at the solution provided by the present invention with a reasonable expectation of success.

Applicants respectfully submit that the activity of the peptides disclosed in Bolognesi et al. is inhibition of HIV transmission to uninfected CD4+ cells, and not inhibition of HIV RT dimerization. Thus the skilled person would have searched for documents of the prior art disclosing methods of inhibiting HIV RT dimerization rather than documents relating to inhibition of HIV transmission to uninfected CD4+ cells. The skilled person would thus not have found the cited Bolognesi et al. document.

In addition, even assuming that the skilled person would have found Bolognesi et al., Applicants do not agree with the Examiner when he asserts that the skilled person would have screened the peptides of the present invention with a reasonable expectation of success. Indeed, said decamer peptides result from an inventive selection of carboxy and amino truncations of the 19-mer peptide disclosed in Divita et al. 1995. Moreover, Bolognesi et al. discloses carboxy or amino truncations of the 36-mer peptide and not carboxy and amino truncations as in the present invention. Thus the skilled person, using the methods disclosed in Bolognesi et al. 1995, would not have found the solution provided by the present inventors.

In consequence, Applicants submit that the subject matter of claims 31-38 is not taught or suggested by the cited references, taken alone or in combination. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At page 6 of the Office Action, claims 39-42 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Divita et al. 1995 in view of Bolognesi et al.; Korber et al., as applied supra to claims 31-38; and Morris et al. (1997).

This rejection is respectfully traversed for at least the reasons set forth above.

Moreover, Applicants note that the Morris et al. reference discloses a new peptide vector, MPG, for efficient delivery of oligonucleotides into mammalian cells. However, Morris et al. does not teach or suggest that the MPG peptide is useful for efficient delivery of another peptide into mammalian cells. In consequence, Applicants do not agree with the Examiner's assertion that it would have been prima-facie obvious to the skilled person to employ the MPG carrier, as provided by Morris et al., to transport antiviral peptides across the cell membrane to facilitate their antiviral activity.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

C. At pages 6-10 of the Office Action, claims 45-53 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Divita et al. in view of Bolognesi et al., Korber et al., and Morris et al.

This rejection is respectfully traversed, for at least the reasons set forth above. Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions related to this response, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at the below-listed telephone number concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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Inhibition of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Dimerization Using Synthetic Peptides Derived from the Connection Domain*

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Based on presently available information on the structure of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase, peptides have been synthesized which correspond to the sequence of a particular region of the protein involved in formation of the active heterodimeric form of the enzyme. Several peptides that are 15–19 amino acids long and that are derived from the so-called connection domain of the reverse transcriptase are able to inhibit dimerization of the enzyme and thus inhibit development of its enzymatic activities. In particular, a tryptophan-rich 19-mer corresponding to residues 389–407 was relatively efficient, showing an apparent dissociation constant in the micromolar range for one or both of the subunits. The sequence of this region is identical for both subunits, since one (molecular mass of 51 kDa) is the proteolytic product of the other (molecular mass of 66 kDa). Dissociation of the preformed heterodimer could not be induced by the peptides, but increasing concentrations reduced the rate of dimerization in a concentration-dependent manner until it became immeasurable at high concentrations. The results suggest that inhibition of dimerization of reverse transcriptase is an attractive approach to chemotherapeutic intervention in HIV infection and that further development of peptide-based inhibition strategies is worth pursuing.

By converting single-stranded genomic RNA into double-stranded proviral DNA, reverse transcriptase (RT)[1] plays a key role in the human immunodeficiency virus (HIV) life cycle. This enzyme consists of two polypeptides of 66 and 51 kDa (Chandra *et al.*, 1986; Di-Marzo Veronese *et al.*, 1986; Lightfoot *et al.*, 1986). The heterodimeric enzyme represents the biologically active relevant form found in infectious virions, whereas the isolated subunits are devoid of enzymatic activities (Müller *et al.*, 1989; Restle *et al.*, 1990, 1992).

RT represents one of the main targets in the development of chemotherapy against HIV, the etiologic agent responsible for the development of acquired immunodeficiency syndrome (AIDS) (Barré-Sinoussi *et al.*, 1983). Inhibitors that have already been developed can be divided into nucleoside and non-nucleoside inhibitors (for reviews, see Grob *et al.* (1992) and De Clercq (1992)). As suggested by Restle *et al.* (1990), the process of dimerization of RT offers an additional interesting target for chemotherapeutic intervention against AIDS. The recent determination by x-ray crystallography of the three-dimensional structure of HIV-1 RT complexed with a non-nucleoside inhibitor (Kohlstaedt *et al.*, 1992) or as a ternary complex with a 19-base/18-base double-stranded DNA template-primer and a monoclonal antibody Fab fragment (Jacobo-Molina *et al.*, 1993) leads to a better understanding of the interaction between the two subunits (p66/p51). The dimer interface, which appears to be highly hydrophobic, is dominated by the interaction of the two so-called connection subdomains (for a review, see Nanni *et al.* (1993)).

Recently, we have used intrinsic tryptophan fluorescence (Divita *et al.*, 1993) and monoclonal antibodies (Restle *et al.*, 1992) to characterize the dimerization process of HIV-1 RT and confirmed that a cluster of 6 tryptophan residues in the connection domains is directly involved in dimer formation, as already proposed (Baillon *et al.*, 1991). Here we present a new strategy against HIV-1 RT activity, namely the use of small peptides corresponding to the connection domains as inhibitors of the dimerization process of the enzyme. Such a strategy has already been applied successfully in the cases of the ribonucleotide reductase from the herpes simplex virus (Cohen *et al.*, 1986; Dutia *et al.*, 1986) and of the protease from HIV-1 (Zhang *et al.*, 1991). In our study, four different peptides corresponding to the connection domain have been tested. Particularly, a peptide corresponding to residues at positions 389–407 of the BH-10 clone derived from the HIV-1 LAV isolate was able to reduce dramatically the rate of dimerization of the enzyme, and appears to be able to interact with one of the subunits. This peptide represents a part of the sequence of the tryptophan repeat motif, which is well conserved in HIV-1 and -2, as well as in simian immunodeficiency virus RTs (Baillon *et al.*, 1991).

EXPERIMENTAL PROCEDURES

Materials—Peptides 1 and 2 were synthesized by Chiron Mimotopes Pty. Ltd. (Clayton, Australia) and provided 95% pure as determined by HPLC analysis. Peptides 3 and 4 were provided by Dr. R. Franck and Dr. H. Gausepohl (ZMBH, Heidelberg, Germany) and were purified by reversed-phase HPLC using an acetonitrile-water linear gradient containing 0.1% trifluoroacetic acid. All peptides were solubilized in a 30% Me_2SO solution at 3.8 mM. The maximal concentration of the peptides in

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[1] The abbreviations used are: RT, reverse transcriptase; HIV, human immunodeficiency syndrome; HPLC, high performance liquid chromatography; LAV, lymphadenopathy-associated virus.

the cell-free assays was 100 μM, which corresponds to a final concentration of less than 1% Me_2SO. It was verified that at this concentration, Me_2SO has no effect on enzyme stability and activity. Acetonitrile (gradient grade) was purchased from Merck. All buffers were filtered and degassed before use.

Enzyme Preparation—Recombinant HIV RTs were expressed in *Escherichia coli* and purified as previously described (Müller *et al.*, 1989). The expression system and purification protocols used allowed the preparation of large quantities of highly homogeneous preparations of the heterodimeric forms of the enzyme resulting from co-expression of the 66- and 51-kDa polypeptides, as well as isolated p51 and p66 subunits. Enzyme concentrations were routinely determined according to Bradford (1976) using a gravimetrically prepared solution of RT as a standard.

Polymerase RT Assay—Polymerase activity was measured by a standard assay using poly(rA)·oligo$(dT)_{15}$ as template-primer (Restle *et al.*, 1990). The RT preparations used showed a specific activity of about 10,000 units/mg, where 1 unit of enzyme catalyzes the incorporation of 1 nmol of TMP in 10 min at 37 °C into acid-insoluble material.

HPLC Size Exclusion Chromatography—Chromatography was performed using two 7.5 × 300-mm HPLC columns in series (Bio-Rad TSK-250, followed by Bio-Rad TSK-125). The columns were eluted with 200 mM potassium phosphate, pH 6.5, at a flow rate of 0.8 ml/min (Restle *et al.*, 1990).

Fluorescence Experiments—Fluorescence measurements were performed at 25 °C, using a SLM-Smart-8000 spectrofluorometer (Colora, Lorch, Germany) equipped with a PH-PC 9635 photomultiplier. The spectral bandwidths were 2 and 8 nm for excitation and emission, respectively. RT excitation was routinely performed at 290 nm, and fluorescence emission intensity was measured at 340 nm. All measurements were corrected for the buffer blank and for the wavelength dependence on the exciting light intensity using the quantum counter rhodamine B in the reference channel. The intrinsic fluorescence emission of RT (0.5–5 μM protein) was measured in a total volume of 0.6 ml of fluorescence buffer containing 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 50 mM KCl, and 1 mM dithiothreitol.

Dissociation and Association of the RT Heterodimer—Dissociation of the HIV-1 RT heterodimer was performed by addition of acetonitrile to a concentration of 17% in the fluorescence buffer described above. The monomer formation was monitored under equilibrium conditions by measuring the relative increase of the intrinsic fluorescence emission of the protein, the decrease of the polymerase activity using the standard assay, or by HPLC size exclusion chromatography (Divita *et al.*, 1993). The association of the subunits was induced by a 12-fold dilution of the sample with an organic solvent-free buffer resulting in a final concentration of 1.4% acetonitrile as previously described (Divita *et al.*, 1993). The establishment of the dimerization equilibrium was followed in a time-dependent manner using the same parameters. For association and dissociation experiments, protein concentrations of 0.5 and 5 μM were used. Data were collected using a personal computer and evaluated with the commercially available fitting program Grafit (Erithacus software). The dimerization experimental data were analyzed as a second order reaction, and the time course of the dissociation of the heterodimer was fitted to a first order equation with a single rate constant.

Antiviral Activity—Peptides 1 and 2 were evaluated for their ability to inhibit HIV-1 infection in cell cultures. The fusogenic effect of HIV-1 in the MT-4 cell line system was determined essentially as described by Rey *et al.* (1987). Briefly, a total of 3×10^5 MT-4 cells in 100 ml of medium (RPMI) were centrifuged, and the pelleted cells were resuspended in 100 ml of RPMI containing increasing concentrations of peptides. After 1 h at 37 °C, the cells were infected by addition of 10 tissue culture infection units in 100 μl of virus HIV-1 LAV. After another hour at 37 °C, the cells were washed three times with RPMI, resuspended in 1 ml of RPMI containing the peptides, and cultured in 24-well culture plates. The appearance of syncytia was followed every day, from day 4 to day 7 post-infection, under an inverted optical microscope. Both uninfected cells and cells infected by the virus in the absence of peptide were used as controls.

RESULTS

Choice of the Peptides—The sequences of the peptides used are defined in Table I (based on the HIV-1 BH10 molecular clone sequence). The recent determination of the three dimensional structure of HIV-1 RT (Kohlstaedt *et al.*, 1992; Jacobo-Molina *et al.*, 1993) provides important information for the design of peptides interfering with the dimerization process of this enzyme. The dimer interface is mainly formed from the interaction between the two connection domains. The marked intrinsic tryptophan fluorescence change induced by dissociation of RT using organic solvent or by subunit association confirmed that the forces involved in this interaction seem to be of highly hydrophobic nature and are stabilized by a cluster of 6 tryptophan residues (Divita *et al.*, 1993). Accordingly, peptides corresponding to the tryptophan repeat motif region of the connection domain were foreseen as potential inhibitors of dimerization of the enzyme. Since the amino acid sequence corresponding to this part of the protein is highly hydrophobic, synthesis of a single peptide encompassing the whole region (amino acids 389–414) was not attempted. Instead, two different partially overlapping peptides were synthesized (Table I). The first one (P1) corresponded to the sequence 389–407, which contained the first 4 tryptophan residues of this region. The last residue, a glutamine, was replaced by a glutamic acid, to improve both the solubility and the purity of the peptide. The presence of a glutamine or glutamic acid residue at the end position of this peptide led to similar inhibition of RT dimerization at similar concentrations. The second peptide (P2) corresponds to residues 403–415, which contain the last 3 tryptophan residues of the cluster. The third (P3) and fourth (P4) peptides corresponded to the middle and the beginning part of the connection domain, respectively .

TABLE I
Amino acid sequence and localization of the different peptides used
Positions are referred to BH10 (molecular clone from HIV-1 LAV).

Peptide	Position	Sequence
1	389–407	FKLPIQKETWETWWTEYWE
2	403–415	TEYWQATWIPEWE
3	330–345	QKQGQGQWTYQIYQEP
4	364–380	DVKQLTEAVQKITTESI

Peptide Effects on the Dimerization Process of HIV-1 RT—As described earlier (Restle *et al.*, 1990; Divita *et al.*, 1993), the heterodimeric form of HIV-1 RT can be reversibly dissociated using organic solvents such as acetonitrile. This results in a complete loss of enzymatic activities without unfolding of the monomers. Both the dissociation and association processes can be followed by three complementary methods: intrinsic fluorescence, HPLC size exclusion chromatography, and enzymatic activity. In the present work, using tryptophan-containing peptides created problems with the fluorescence method, so that the association of the subunits was followed by measuring the polymerase activity and the final dimeric ratio was shown to be 100% by size exclusion HPLC (data not shown). In the experiments shown in Fig. 1, HIV-1 RT heterodimer was dissociated first and then reassociated as described under "Experimental Procedures," using a fixed peptide concentration of 10 μM. In the presence of P1, the apparent RT association rate constant (k_a) was greatly reduced (to 0.2×10^2 M^{-1} s^{-1}, compared to 3.3×10^2 or 5×10^2 M^{-1} s^{-1} in the case of P2 or in the standard conditions in the absence of peptide.) Half-time values ($t_{1/2}$; *i.e.* the time needed to reestablish 50% of final activity) of 2, 3, and 50 h were determined in the absence of peptide or presence of P2 and P1, respectively, at the same concentration. After 200 h, only 80% of the dimeric form was restored in the presence of P1. Similar results were obtained with the association of separately purified subunits (p66 and p51), indicating that the acetonitrile treatment did not affect subunit association or its inhibition by peptides.

The peptides P3 and P4, which were not derived from the tryptophan cluster of the connection domain, also inhibited the association process, but less profoundly than P1. Values for k_a

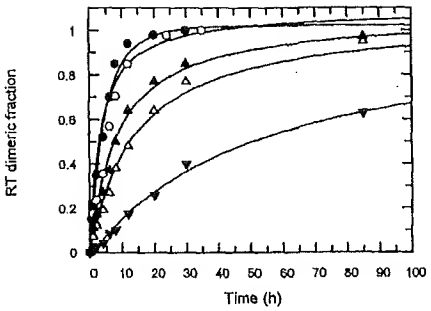

FIG. 1. **Peptide inhibition of the RT dimerization process.** The heterodimer was dissociated with 17% acetonitrile, and reassociation was initiated by lowering the acetonitrile concentration to 1.4%. All the reactions were performed at 25 °C, with a RT concentration of 0.25 μM, in the presence of 10 μM P1 (▼), P2 (○), P3 (△), or P4 (▲), or in the absence of peptide (●). The dimeric RT form was estimated from the polymerase activity, and the data were fitted using a second order equation.

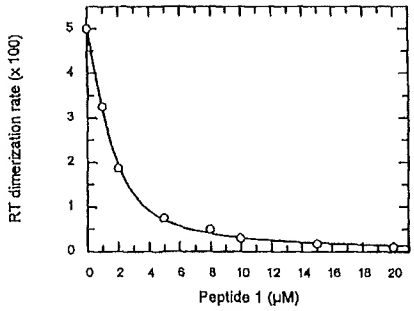

FIG. 2. **Dependence of the apparent second order rate constant for RT dimerization (k_a) on the concentration of P1.** RT was dissociated and reassociated as described in Fig. 1, in the presence of increasing P1 concentration. The dimerization rate constant was determined by fitting the time-dependent association curve with an equation for describing a second order reaction.

of 0.8×10^2 and 1.2×10^2 M^{-1} s^{-1} and for $t_{1/2}$ of 12 and 8 h were obtained for P3 and P4, respectively (Fig. 1).

Fig. 2 shows a correlation between P1 concentration and the rate of dimerization of HIV-1 RT. Essentially complete inhibition was observed for a concentrations of 25 μM or above, and an apparent dissociation constant for this peptide was estimated to be 1.2 μM. In order to limit a possible effect of Me_2SO, in which the peptides were dissolved, a value of 10 μM of each peptide was arbitrarily chosen for the following experiments in which this peptide was used. At this concentration P1 produced an 80% reduction in the rate of dimerization.

At the concentrations used (10 μM), none of these peptides actively induced RT dissociation or modified the polymerase activity of the heterodimer of HIV-1 RT. This result was not surprising, due to the very low apparent equilibrium dissociation constant (K_d) of 4×10^{-10} M for the heterodimeric HIV-1 RT (Divita *et al.*, 1993), in comparison with the K_d of 1.2 μM for P1, the most active of the peptides used. Spontaneous dissociation of HIV-1 RT is also very slow.

HIV-1 RT dissociation achieved using 17% acetonitrile was followed by intrinsic fluorescence, as described previously (Divita *et al.*, 1993). In the presence of 10 μM P1, the rate of dissociation was slightly increased from 4.5×10^{-3} s^{-1} in the standard condition to 7.1×10^{-3} s^{-1}. In contrast, the three other peptides tested did not alter the dissociation rate.

Competition between P1 and Isolated Subunits—Direct interaction of P1 with RT subunits was further analyzed by competition experiments using purified isolated p51 or p66 subunits (Fig. 3). The competition experiments were performed by increasing the concentration of individual subunits and using fixed concentrations of heterodimeric RT (0.5 μM) and peptide (10 μM). The RT association process was followed by measuring the polymerase activity and checked by HPLC size exclusion at final time values. In both cases, the P1 inhibition was weakened by the presence of an excess of subunits. Values of 0.5 and 1.1 μM were obtained for half-maximum effects, using p51 and p66, respectively. The presence of p51/p51 homodimers was not detected, which is consistent with the low stability of this homodimer in the concentration range used (Restle *et al.*, 1990). In contrast, for high concentrations of p66 subunits, 15% of the p66/p66 homodimeric form was detected by HPLC size exclusion (data not shown). This might explain the slightly lower competitive effect of p66 compared to that of p51 on the association process in the presence of P1.

Peptide Combinations—The combination of different peptides has been tested on the inhibition of the RT association process (Fig. 4). The combination of P1 with P3 or P4 increased the extent of inhibition by a factor of ~2, with k_a values of 13.3 and 9.3 M^{-1} s^{-1}, respectively. In both cases, the maximal fraction of dimeric RT restored after 200 h, was only 65%, suggesting a strong interaction between the peptides and the subunits. In contrast, P2 induced no additive effect on the P1 inhibition. None of the peptide combinations can induce significant RT heterodimer dissociation under our experimental conditions.

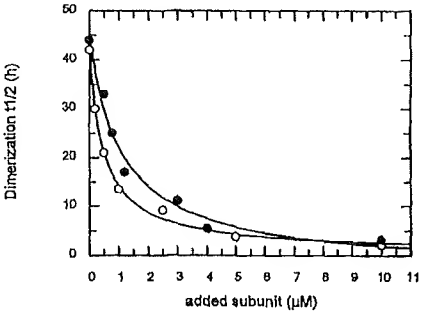

FIG. 3. **Competition between P1 and isolated subunits P51 and p66.** The RT association was made in the presence of fixed P1 concentration (10 μM), and increasing concentration of P51 (○) or P66 (●).

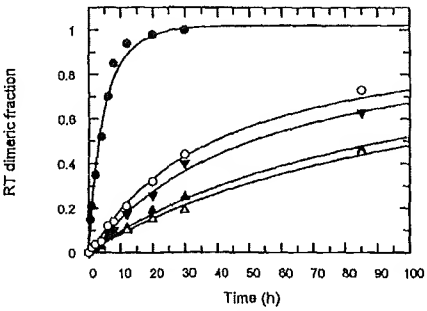

FIG. 4. **Effect of peptide combination on the RT dimerization process.** Experiments were performed as described in Fig. 1, in the presence of 10 μM P1 + P2 (○), P1 + P3 (▲), P1 + P4 (△), or P1 (▼), or in the absence of peptide (●) as control.

Effect of Peptides on Viral Replication—The effect of P1 and P2 on HIV-1 replication in MT-4 cells was determined as described under "Experimental Procedures." After 7 days of culture in the presence of 50, 100, or 200 μM P1 or P2, no protection of the cells was detected, as measured by syncytia formation, when compared to the culture with the virus and no peptide (not shown). It was verified that the peptides have no effect at the concentrations used on MT-4 cell growth in the absence of infection.

DISCUSSION

In this paper, we describe for the first time a new class of RT inhibitors, which correspond to synthetic peptides derived from the connection domains of HIV-1 RT. Three peptides corresponding to the sequences 331–345, 364–380, and 389–407 of the RT from HIV-1 are able to interfere *in vitro* with the dimerization process of this enzyme.

The most dramatic effect was obtained with a peptide (P1) containing the β-strand 19 and the α-helix L from the connection domains, as defined in the RT three-dimensional structure (Kohlstaedt *et al.*, 1992; Jacobo-Molina *et al.*, 1993). P1 showed a relatively high affinity for the subunits (1.2 μM) and reduced by 20-fold the rate of dimerization at 10 μM. The results obtained do not allow a decision concerning the exact mechanism of the inhibition to be made. The data of Fig. 3 can be explained by binding of P1 to p51, to p66, or to both subunits.

The lack of additive effect of P2 on P1 inhibition of the reassociation process is not surprising if one considers that the two peptides overlap by 5 residues. This, together with the fact that P2 did not influence RT dimerization suggests that only the first part of the tryptophan cluster is directly involved in the association between p66 and p51 subunits. The significant inhibition of the dimerization process by the peptides P3 and P4 and the powerful inhibition of dimerization of HIV-1 RT *in vitro* by combination of P1 with these peptides suggests that the α-helix K and the β-strands 16 and 17 are also critical in the dimer formation.

Preliminary results suggest that P1 is not able to inhibit HIV replication in cell culture. This is not surprising, since it is unlikely that a peptide of this size can penetrate cells without degradation. Further work is needed to test this point, but we are presently considering two possible approaches to overcome this problem. The first of these is chemical modification of the peptide with the aim of increasing its stability to proteases and its chances of crossing the cell wall. This approach has worked well for active site inhibitors of HIV-1 protease but may be more difficult to realize in the present case, due to the greater length of peptide needed for a significant inhibitory effect. The second approach, which is perhaps more interesting and promising in the longer term, is the intracellular expression of peptides that act as inhibitors of the dimerization reaction. There is no principal limitation on the length of peptides in this approach. Indeed, it is probably advantageous to use a longer peptide, which can form an independent folding unit with an analogous structure to that which it assumes in the native form of the enzyme. Choice of potential sequences could be either "rational," *i.e.* based on a more detailed knowledge of the structure of the heterodimer, or perhaps more promisingly could be based on a selection procedure using, for example, a library of deletion mutants. The selection procedure would have to choose on the basis of stable interaction with one of the subunits without inducing enzymatic activity.

We expect that peptides of the type described here must be highly selective for their target proteins. There is, for example, no reason to expect interaction with cellular polymerases, since their overall structure is in general different to the unusual heterodimeric structure of the immunodeficiency RTs. Interestingly, P1 also inhibits dimerization of HIV-2 RT,[2] which has a highly homologous sequence to HIV-1 RT in this region of the connection domain, in particular with respect to the aromatic cluster.

The present work serves to identify a principle approach for the inhibition of RT dimerization and to establish methods for quantitating these effects. Further work will be dedicated to designing or selecting peptides or truncated proteins of greater potency than the peptides identified here. Further development of vectors for targeted gene transfer could then result in intracellular expression of such sequences for chemotherapy or prevention of AIDS, the latter then being an example of intracellular immunization, as defined by Baltimore (1988).

Acknowledgments—We thank Ivan Hirsch for stimulating discussions and Evelyne Doriat for the cell culture experiments. We also thank Dr. Rainer Frank and Dr. Heinrich Gausepohl for the synthesis of the HIV-1 RT peptides 3 and 4.

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